GEORGIA INSTITUTE OF TECHNOLOGY — OFFICE OF CONTRACT ADMINISTRATION

PROJECT ADMINISTRATION DATA SHEET

[X] ORIGINAL [ ] REVISION NO. _____

Project No. G-32-626 (R6117-1A0) GTRC/GIT DATE 04/14/86

Project Director: D.H. Hall School/Lab Biol.

Sponsor: DHHS/PHS/NIH/NIGMS

Type Agreement: Grant No. 1R01-GM36714-01

Award Period: From 4-1-86 To 3-31-87 (Performance) 6-31-87 (Reports)

Sponsor Amount:	This Change	Total to Date
Estimated: $		$ 80,382
Funded: $		$ 80,382

Cost Sharing Amount: $ 4,653 Cost Sharing No: G-32-341 (F6117-1A0)

Title: Genetics of the Intron-Containing TD Gene of Phage T4

ADMINISTRATIVE DATA OCA Contact John R. Schonk X-4820

1) Sponsor Technical Contact:

Dr. Barbara R. Williams
National Institutes of Health
National Institute of General Medical Sciences
Grant Management Office
Bethesda, MD
~~301/496-7087~~

2) Sponsor Admin/Contractual Matters:

Dona McNish
National Institutes of Health
National Institute of General Medical Sciences
Grants Management Office
Bethesda, MD
301/496-7166

Defense Priority Rating: N/A Military Security Classification: N/A

(or) Company/Industrial Proprietary: N/A

RESTRICTIONS

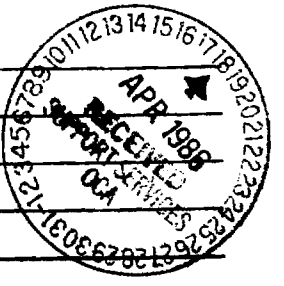
See Attached NIH Supplemental Information Sheet for Additional Requirements.

Travel: Foreign travel must have prior approval – Contact OCA in each case. Domestic travel requires sponsor approval where total will exceed greater of $500 or 125% of approved proposal budget category.

Equipment: Title vests with GIT

COMMENTS:

No Funds may be expended after 3/31/87



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FORM OCA 4:383

GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

Date 5-22-87

Project No. G-32-626 School/~~Lab~~ Applied Biology

Includes Subproject No.(s) N/A

Project Director(s) D.H. Hall GTRC / ~~GIT~~

Sponsor DHHS/PHS/NIH/NIGMS

Title Genetics of the Intron-Containing TD Gene of Phage T4

Effective Completion Date: 3/31/87 (Performance) 6/31/87 (Reports)

Grant/Contract Closeout Actions Remaining:

- [ ] None
- [X] Final Invoice or Final Fiscal Report
- [ ] Closing Documents
- [ ] Final Report of Inventions
- [ ] Govt. Property Inventory & Related Certificate
- [ ] Classified Material Certificate
- [ ] Other

Continues Project No. Continued by Project No. G-32-639

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Other Duane H.
Angela DuBose
Russ Embry

FORM OCA 69.285

SECTION IV PROGRESS REPORT SUMMARY	GRANT NUMBER GM36714-02 G-32-626	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Dwight H. Hall	PERIOD COVERED BY THIS REPORT FROM 4/1/86	THROUGH 1/27/87
NAME OF ORGANIZATION Georgia Institute of Technology		

TITLE *(Repeat title shown in item 1 on first page)*
Genetics of the Intron-Containing td Gene of Phage T4

(SEE INSTRUCTIONS)

Publications

Hall, D.H., C.M. Povinelli, K. Ehrenman, J. Pedersen-Lane, F. Chu and M. Belfort, 1987. Two domains for splicing in the intron of the phage T4 thymidylate synthase (*td*) gene established by nondirected mutagenesis. *Cell, 48*: 63-71.

Hall, D.H., C.M. Povinelli, K. Ehrenman, J. Pedersen-Lane and M. Belfort, 1986. Two splicing domains in the intron of the phage T4 *td* gene established by non-directed mutagenesis. *Abstracts of Papers Presented at the Meeting on Molecular Genetics of Bacteria and Phages* (Cold Spring Harbor Laboratory, NY): 171.

Hall, D.H., C.M. Povinelli, K. Ehrenman, J. Pedersen-Lane and M. Belfort, 1986. Intron mutations in the T4 gene coding for thymidylate synthase. *Abstract, Fed. Proc.* (FASEB) *45*: 1703.

Hall, D.H., C.M. Povinelli, K. Ehrenman, J. Pedersen-Lane and M. Belfort, 1986. Non-directed splicing-defective mutations in the intron of the phage T4 *td* gene. *Abstracts of Papers Presented at the Meeting on RNA Processing* (Cold Spring Harbor Laboratory, NY): 131.

Belfort, M., J. Pedersen-Lane, K. Ehrenman, D.H. Hall, C.M. Povinelli, J.M. Gott and D.A. Shub, 1987. Processing and genetic characterization of self-splicing introns of bacteriophage T4. *Molecular Biology of RNA: New Perspectives* (Academic Press, Inc.). (in press)

Brown, M.D., D.H. Hall, C.M. Povinelli, K. Ehrenman, J. Pedersen-Lane, and M. Belfort, 1987. Mutations affecting RNA splicing in the phage T4 thymidylate synthase gene. *Abstracts of the Annual Meeting of the American Society for Microbiology*. (in press)

Report

1. There has been no change in the general scientific goals of the project.

2. We have been genetically mapping and biochemically characterizing many mutations affecting the expression of the thymidylate synthase and dihydrofolate reductase genes. Our main results are summarized in the following abstract of a presentation given at the 1986 meeting on Molecular Genetics of Bacteria and Phages at the Cold Spring Harbor Laboratory in New York.

> TWO SPLICING DOMAINS IN THE INTRON OF THE PHAGE T4 *td* GENE ESTABLISHED BY NON-DIRECTED MUTAGENESIS. Dwight H. Hall*, Christine M. Povinelli*, Karen Ehrenman**", Joan Pedersen-Lane** and Marlene Belfort**. *School of Applied Biology, Georgia Tech, Atlanta, GA 30332; **Wadsworth Laboratories, N.Y. State Dept. of Health, Albany, NY 12201; "Dept. of Microbiology and Immunology, Albany Medical College, Albany, NY 12208.
>
> The T4 gene (*td*) coding for thymidylate synthase contains a group I intron similar to the intron in the *Tetrahymena* self-splicing rRNA. We are using the T4 *td*

SECTION IV - PROGRESS REPORT SUMMARY (Continued)

gene for genetic analysis of a group I intron. A random saturation mutagenesis approach directed against the intact phage genome was facilitated by rapid phenotypic screening methods for T4 *td* phage. These non-directed mutations are being localized by recombinational mapping using marker rescue in cells containing defined *td* subfragments. Among 97 nitrous acid or hydroxylamine induced *td* mutations that have been localized, 27 map to the intron. None of the mutations are in the middle of the 1017 nucleotide (nt) intron, as defined by a 635 nt deletion. Both this deleted *td* gene, which is splicing proficient, and the point mutations indicate that the functional domains for splicing in the intron are located within 200 nt of the two intron-exon boundaries.

The base change has been determined for one 5' (T4 *td*N57) and one 3' mutant (T4 *td*N47). N57 is a C to T transition 10 nt from the 5' splice site in the putative internal guide sequence, while N47 is a G to A transition 49 nt from the 3' splice site in a region of potential secondary structure. Whereas N57 results in greatly reduced levels of cleavage at the 5' splice site, N47 causes both diminished and inaccurate cleavage at this site. A molecular consequence of the 3' N47 mutation therefore appears to be manifested almost 1,000 nt upstream, at the 5' splice site, probably reflecting disruption of the secondary structure of the intron. Further analysis of these and other mutants in the collection will, without any *a priori* structure-function assumptions, delineate those sequences important in the group I splicing pathway.

Our studies are providing new types of T4 mutants affecting RNA splicing. Characterization of these mutants will lead to a better understanding of the mechanism and the role of RNA splicing in T4-infected *E. coli*. A knowledge of the mechanisms involved in RNA splicing in phage-infected bacteria should be helpful in the analysis of RNA splicing in eukaryotes. It is likely that in some cases very similar mechanisms will be found.

3. The specific objectives for the coming year are:

   a. to further characterize more *td* and *nrd* mutations from our collection genetically and biochemically, especially those that affect RNA splicing, and

   b. to isolate and characterize false revertants of *td* and *nrd*B mutants defective in RNA splicing.